Use of immunoblot assay to define serum antibody patterns associated with *Helicobacter pylori* infection from Bahrain

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Helicobacter pylori is recognized as an important human pathogen that causes chronic gastritis and is associated with gastric atrophy which can lead to adenocarcinoma and MALT lymphoma of the stomach [1,2]. The establishment of the *H. pylori* infection in the stomach depends on several host and bacterial factors [1]. The expression of specific host antigens such as Lewis-type blood group antigen, may play an important role [3,4]. *Helicobacter pylori* itself secretes several enzymes, such as urease, which, by neutralizing the acidic pH of the stomach allows the organism to survive [1].

Among the most important bacterial factors in the pathogenesis are the CagA protein and the VacA cytotoxin. The presence of CagA is associated with duodenal ulceration, gastric mucosal atrophy and gastric cancer [5]. Another attribute is the cytotoxin that induces the formation of vacuoles in mammalian cells *in vitro* leading to cell death [6]. This toxin is encoded by the *vacA* gene which appears to be highly diverse, and recently the existence of a different allelic variant in the second segment of this gene has been described [7,8].

The serum antibody response could provide important information regarding the severity of *H. pylori*-associated disease. Several studies have demonstrated a strong correlation between the level of anti-*H. pylori* immunoglobulin (Ig)G antibodies and the colonization of the gastric mucosa by bacteria [1,9]. However, the *H. pylori* IgG antibody patterns have been reported to show a high degree of diversity [10,11]. Xiang et al. [12] described an enzyme immune assay (EIA) with recombinant antigen including a fragment of the CagA protein. They concluded that there is a positive correlation between EIA and Western blotting methods used to detect anti-CagA antibodies. They have also shown a strong correlation between CagA antibody level and the presence of an ulcer. Other studies have found that Western blot is even more sensitive as well as more specific than EIA [11,12]. However, other antibodies or combinations of antibodies may also be useful as markers of the severity of the disease.

The present study was undertaken to determine the prevalence of antibodies to Cag A, and VacA in our patients, and also to study whether the infection with an *H. pylori* strain expressing the *vacA* and/or the *cagA* is associated with severe disease outcome in our Bahraini patient population.

A total of 57 consecutive patients examined in the Gastro-Enterology Clinics in Bahrain, were enrolled in the study between 1997 and 1998. The patients presented with dyspeptic syndromes and underwent an upper gastroduodenal endoscopy. All the patients were seropositive by total IgG EIA (Meddens Diagnostics B V, Vorden, The Netherlands). They had received no antimicrobial therapy during the previous 2 months. Sera were collected on the day of the endoscopy; they were aliquoted and frozen at $-80\,^{\circ}C$ until they were used.

The CagA and VacA antibodies were determined by commercially available kits, the RIDA Blot *Helicobacter* (R Biopharm GmbH, Hamburg, Germany), a qualitative *in vitro* immunoblot test for the detection of specific antibodies (IgA, IgG) against *Helicobacter pylori* in human serum and plasma, were used in this study.

Samples were evaluated by using evaluation strip template (coated with antigen bands of *H. pylori*) lined with sample bands. These bands received point values which are determined depending on the meaning of the bands and their detected immunoglobulin class. The results of IgG and IgA were classified as positive, questionable or negative according to the manufacturer's recommendations.

The chi-square test was employed to evaluate the results by using a statistical analysis program (SPSS Microsoft 7.5 for windows). The level of significance was established for *P*-values < 0.005.

All 57 patients underwent gastroscopy. Twenty-eight (49%) were suffering from moderate to severe gastritis, seven (12%) had duodenal ulcer, four 4 (7%) had duodenitis and 18 (31%) were normal by endoscopy.

The analysis of patients serum by immune blot using the kit scoring systems showed that 47 (82%) of our patients were positive for IgG, nine (15%) were negative and one (3%) questionable. Only 26 (45%) were positive with the IgA test, 25 (45%) were negative and six (10%) questionable (Table 1). From these results one can clearly conclude that the number of positive *H. pylori* cases were significantly more reactive with

Table 1 Total Immunoblot results of IgG and IgA tests

Results	IgG Test		IgA Test	
	No. of cases	(%)	No. of cases	(%)
Positive	47	82	26	45
Not known	1	1.7	6	10.5
Negative	9	15	25	43.8

the IgG test than with the IgA test with a mean rank of 15.02 and a significance rate of $P < 0.001$.

A total of 57 blots of patients' serum were analysed: the blots revealed from 1 to 15 bands with an average of 7.2 bands for the IgG tests and 1 to 7 bands with an average of 1.9 bands for IgA. The blots were analysed by more than one investigator. Fifteen different bands were distinguished on the 57 blots.

A high percentage of our patients' sera were reactive to the 29 and 28 kDa bands; 45 (78%) and 46 (80%), respectively.

We analysed the blots for the presence of CagA, and VacA antibodies. Thirty-two (56%) of our patients developed IgG antibodies to CagA and VacA. One developed antibodies to CagA only, three to VacA only and 21 patients did not develop antibodies to either.

Twenty-two (78.5%) patients with gastritis were positive for CagA and 23 (82.1%) were positive for VacA antibodies, compared with only five (27.7%) and six (33.3%), respectively, of the subjects with normal endoscopy ($P < 0.005$). The seven patients with duodenal ulcer were positive for both CagA and VacA antibodies.

Several methods for laboratory diagnosis of *H. pylori* have been described, which fall within two categories. The bacteriological (culture method) is unquestionably the most specific, but it is subject to sample errors and not very sensitive. The other category is the breath test and serology; they are more sensitive, but they may be less specific. Serology is now considered to be a global method of diagnosis with a variety of commercially available kits, most of them providing satisfactory reactions [1,9,13,14]. However significant improvement must be made before it can be considered as a reference method. Our data are in agreement with other studies [15,16] with regard to the importance of IgG in the immunodiagnosis of *H. pylori* infection when compared to IgA. This may be due to the fact that IgA antibodies may appear earlier than IgG during the infection period and disappear after a short time (short half life) [17] whereas IgG antibodies may persist for a long time in the serum.

In our study we found four antigens 120, 87, 29, 28 kDa to be the most immunogenic during *H. pylori* infection in our patient population, which is consistent with other studies [11]. However a large number of our patients' sera (78%) were reactive to the 29 kDa band (urease A), and 80% were reactive to an unknown 28 kDa band. The reason for this high percentage needs to be investigated.

Helicobacter pylori infection leads to a variety of diseases. At present the only reliable way to identify the association of *H. pylori* with the illness remains endoscopy in combination with histological examination of the gastric mucosa. Large numbers of studies were conducted to correlate the severity of the *H. pylori* infection to the antibody level, specificity and type [2,18–21]. In our patient population we found that both anti-Cag A, and -VacA existed more frequently in patients with gastritis and duodenal ulcer, which is also consistent with the above studies. Eighteen (31%) of our patients were normal by endoscopy, despite being positive by total IgG EIA. This could be due to the fact that peptic ulcer or gastritis may be intermittent and some patients may be non-ulcerous at the time of endoscopy.

An elevated proportion of the sera from symptomatic Bahrain patients showed IgG antibodies against CagA and VacA antigens. This proportion was higher among patients with gastritis or ulcer disease in comparison with the subjects with normal endoscopy. Immunoblot would be useful for screening patients at risk of ulcer or even before starting therapy.

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Clearance of a fluconazole-resistant *Candida albicans* strain after switching antifungal therapy and initiation of triple therapy for HIV infection

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Oropharyngeal candidiasis (OPC) that is refractory to therapy with fluconazole has been increasingly reported in HIV-infected patients after long-term use of fluconazole [1]. Recently, a patient was reported in whom fluconazole-refractory mucosal candidiasis resolved after the initiation of treatment with an antiretroviral agent combined with a protease inhibitor [2]. It was argued that improving immune function was responsible for this effect, but information about the *Candida* strains was not given. In this report, we describe a patient who suffered from fluconazole-refractory oral and esophageal candidiasis (OC) but resolved this disease after a change in antifungal therapy as well as initiation of highly active antiretroviral therapy (HAART), together with clearance of the fluconazole-resistant *C. albicans* strain.

A 37-year-old homosexual man with HIV infection diagnosed in 1993 presented in March 1996 with a gastric lymphoma and OC. The CD4 count was 2/μL, and the HIV load was 32×10^3 copies RNA/mL. He had a history of recurrent OPC since 1994 and had received daily treatment with fluconazole 200–400 mg/day for the past 2 years. The Epstein–Barr virus-associated lymphoma in the stomach was treated with chemotherapy and resolved in May 1996. During this period, OPC and OC persisted despite administration of fluconazole (200–400 mg/day) and amphotericin B oral suspension (12 mL/day). In November 1996, intravenous therapy with Ambisome (5 mg/kg/day) was given and candidiasis resolved temporarily, but reoccurred rapidly under secondary prophylaxis with Ambisome (5 mg/kg/day) given once-weekly in January 1997. Antiretroviral therapy was changed from single therapy with saquinavir in November 1996 to triple therapy (HAART) with zidovudine (250 mg twice-daily), lamivudine (150 mg twice-daily) and saquinavir (600 mg thrice-daily). Due